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Selective loss of dopamine D_3-type receptor mRNA expression in parietal and motor cortices of patients with chronic schizophrenia

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ABSTRACT **The expression of dopamine D_3-subtype-receptor mRNA was analyzed in defined anatomic regions of brain obtained postmortem from patients with chronic schizophrenia and from controls. The specific amplification of D_3-encoding cDNA by PCR allowed the identification of D_3 mRNA expression in a wide variety of anatomic regions in both control brains and brains obtained from schizophrenic patients. However, in the parietal cortex (Brodmann areas 1, 2, 3, and 5) and motor cortex (Brodmann area 4), a selective loss of D_3 mRNA expression was found in schizophrenia. A different D_3 mRNA species was identified that appears to be widely expressed and that is still found in those regions of schizophrenic brains where D_3 mRNA could not be detected. Compared with D_3 mRNA this RNA is significantly less abundant, and at present its function (if any) is unclear. Many variables associated with either the course and/or the therapeutic management of the disease may account for the selective loss of D_3 mRNA in the motor, somatosensory, and somatosensory association areas of schizophrenic brains.**

The recent cloning of several related cDNAs and genes that code for dopamine-receptor proteins has identified three distinct subtypes that have a transmembrane topology typically found in the primary structure of members of the superfamily of G protein-coupled receptors. These subtypes are designated as D_1 and D_1-like (1–6), D_2 and D_2-like (7–11), and D_3 (12). Like the gene that codes for the D_2 subtype receptor, the D_3 subtype-receptor-encoding gene contains introns, and the encoded protein is a target for typical and atypical neuroleptics with proven antipsychotic efficacy. However, the D_3 subtype receptor differs from other members of the dopamine-receptor family in its anatomic distribution, which is associated with limbic areas that control cognitive and emotional aspects of behavior, and perhaps also in its signal-transduction mechanisms (12, 13).

Because alterations in dopamine D_3-receptor expression or function may be involved in certain psychopathologies, we have analyzed the expression of mRNAs encoding D_3 receptors in a variety of anatomic regions of postmortem brains from patients with chronic schizophrenia and from controls. This led to the identification of a different D_3 mRNA species, named D_{3nf},§ which encodes a protein that differs in the carboxyl terminus from the originally reported human D_3 receptor (14). If both mRNAs are indeed translated into proteins, it is conceivable that D_{3nf} does not function as a G protein-coupled receptor. Despite the low abundance of D_{3nf} mRNA, we could detect its expression in all brain regions examined. In contrast, the expression of D_3 mRNA was found to be selectively lost in the motor and parietal cortex of patients with chronic schizophrenia.

MATERIALS AND METHODS

RNA Isolation, PCR Amplification of cDNAs, Southern Blotting, and Nucleotide Sequencing. RNA was extracted as described by Chirgwin *et al.* (15). For studies on human brain tissues, a region of the parietal cortex that corresponds to Brodmann areas 1, 2, 3, and 5 and a region of the motor cortex (Brodmann area 4) were extensively examined. For the synthesis of first-strand cDNA, 5 μg of total RNA was primed with $(dT)_{15}$ and incubated at 37°C for 1 hr with 200 units of Moloney murine leukemia virus reverse transcriptase (United States Biochemical). For subsequent PCR amplification (94°C, 30 sec; 52°C, 1 min; 72°C, 2 min; 35 cycles) of specific cDNAs, the following synthetic oligonucleotide primer pairs were used: (*i*) D3S5′: 5′-TACCTGCCCTTTGGAGT-3′/D33′: 5′-CTCCCTCAGCAAGACAG-3′. These primers direct amplification of the carboxyl-terminal half of the cDNA that codes for the human D_3 receptor and extend from the 3′ end of the putative transmembrane-spanning domain 5 to the 3′ end (see ref. 14). (*ii*) D35′: 5′-ATGGCATCTCTGAGTCAG-3′/D3B3′: 5′-TCCCGAAGTGGCACTCA-3′. This primer pair directs amplification of the 5′ portion of the D_{3nf}-specific cDNA. The primer D3B3′ contains a sequence found only in D_{3nf} (see Fig. 1*B*), and D35′ recognizes the beginning of the coding region of D_3 (see Fig. 3). (*iii*) D35′/RPD3: 5′-CAGCTTCAAAGATGTCG. This primer pair directs amplification of the 5′ portion of D_3-specific cDNA. The primer RPD3 recognizes a sequence in the carboxyl-terminal end of the third cytoplasmic domain that is found in D_3 mRNA but is not found in D_{3nf} (see Fig. 3). (*iv*) D35′: 5′-ATGGCATCTCTGAGTCAG-3′/D33′: 5′-CTCCCTCAGCAAGACAG-3′. These primers direct amplification of the full-length coding region of the human D_3-receptor cDNA according to the sequence reported in ref. 14. (*v*) D3S5′(2): 5′-ACTCGGAATTCCCTGAG-3′/D3S(2R): 5′-TTGCCTTCTTCTCCCGA-3′. These primers were used for PCR amplification of the genomic locus of the D_3-encoding gene that contains sequences encoding the carboxyl-terminal half of the putative third cytoplasmic domain of the receptor (see Fig. 3).

For PCR 2.5 units of *Taq* polymerase (Promega) were used. PCR products were cloned into the plasmid vector PCR_{1000} (Invitrogen), and the nucleotide sequence of their single strands was obtained in both orientations by the dideoxynucleotide chain-termination method (16).

For Southern blots, PCR products were separated on a 1% agarose/Tris/acetic acid/EDTA gel and subsequently transferred to Zeta-Probe (Bio-Rad) membranes by the method of alkaline transfer (17). Blots were hybridized to a random-primed (18) ^{32}P-radiolabeled cDNA that codes for the human D_3 receptor at 68°C in a buffer containing 10% Blotto, 1% SDS, 5× standard saline/citrate (SSC), 5× Denhardt's so-

Abbreviations: AD, Alzheimer disease; PMI, postmortem intervals.
‡To whom reprint requests should be addressed.
§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L20469).

lution, 0.05 M sodium phosphate, and 5 mM EDTA. Blots were washed at 68°C in a buffer containing 0.1× SSC and 1% SDS.

Tissue Sources. Postmortem brain tissues were obtained through the Brain Bank Facilities of the Department of Psychiatry at Mount Sinai Medical School (New York). The tissues were dissected without knowledge of diagnostic classification. The schizophrenic brain tissues were obtained from consecutive autopsies of chronically ill and long-term-hospitalized individuals who died at a state-supported long-term psychiatric hospital. The Diagnostic and Statistical Manual of Mental Disorders III-R diagnosis of schizophrenia was based on a retrospective review of all medical charts. None of the schizophrenic patients had histological findings compatible with a diagnosis of Alzheimer disease (AD). The 18 schizophrenic patients examined here were classified as chronic paranoid (n = 8), disorganized (n = 4), undifferentiated (n = 4), and catatonic schizophrenia (n = 2). Tissues classified as AD were obtained from patients who died in hospitals and nursing homes of the New York area and whose medical histories demonstrated progressive deterioration of cognitive functions. All AD tissues met the neurohistological criteria for AD. Control brains were obtained postmortem from individuals with no history of psychotic disorders and no histological findings compatible with the diagnosis of AD who died in hospitals or nursing homes of the New York area.

All three groups have mixed ethnic backgrounds. The ages of these patients at death (mean ± SD) were as follows: 76 ± 13.2 yr, schizophrenia (n = 18; 11 females, 7 males); 70 ± 8.9 yr, AD (n = 9; three females, six males); and 74 ± 12.7 yr, controls (n = 9; five females, four males). The postmortem intervals (PMIs; time between death and freezing of tissues; mean ± SD) were 321 ± 121 min for the first nine samples from schizophrenic patients (Fig. 4) and varied for the remaining nine samples between 216 min and 1640 min. The PMIs for AD cases were 281 ± 94.1 min and for controls were 289 ± 165 min.

RESULTS

Initially, experiments were designed to amplify the cDNA encoding the carboxyl-terminal half of the human dopamine D_3 receptor by PCR. The amplified sequence extends from the 3′ end of the fifth transmembrane-spanning domain to the 3′ end of the coding region. It includes the putative third cytoplasmic domain, which is thought to be a major determinant of G protein coupling (19). cDNAs from human postmortem brain tissue and from the human neuronal cell line SY5Y were used as a template for PCR amplification specified by the primer pair D3S5′/D33′ (see *Materials and Methods*). In addition to the expected D_3-specific PCR product of 618 nt in length, a second product was obtained from both tissue and cell line templates that was ≈100 nt shorter. Both products hybridized to a ^{32}P-radiolabeled D_3-corresponding cDNA on a Southern blot probed under high-stringency conditions (Fig. 1*A*). The nucleotide sequence of the 618-nt-long product was found to be identical to the analogous sequence of the D_3 receptor published previously (14). The nucleotide sequence of the shorter product, D_{3nf}, was identical to the D_3-specific product except for a deletion of 98 nt that encoded the carboxyl terminus of the putative third cytoplasmic domain of the D_3-specific sequence (ref. 14; Fig. 1*B*).

Although amplification of both D_3- and D_{3nf}-specific fragments was obtained from cDNAs of parietal cortical tissues from control brains, cDNAs obtained from parietal cortical tissues of postmortem schizophrenic brains allowed only the D_{3nf}-specific amplification (Fig. 1*C*, lane 2). Therefore, such cDNAs could be used to generate a full-length open reading frame of D_{3nf}. When a D_{3nf}-specific sequence (Fig. 1*B*) was used as a 3′ primer in conjunction with the 5′ primer D35′

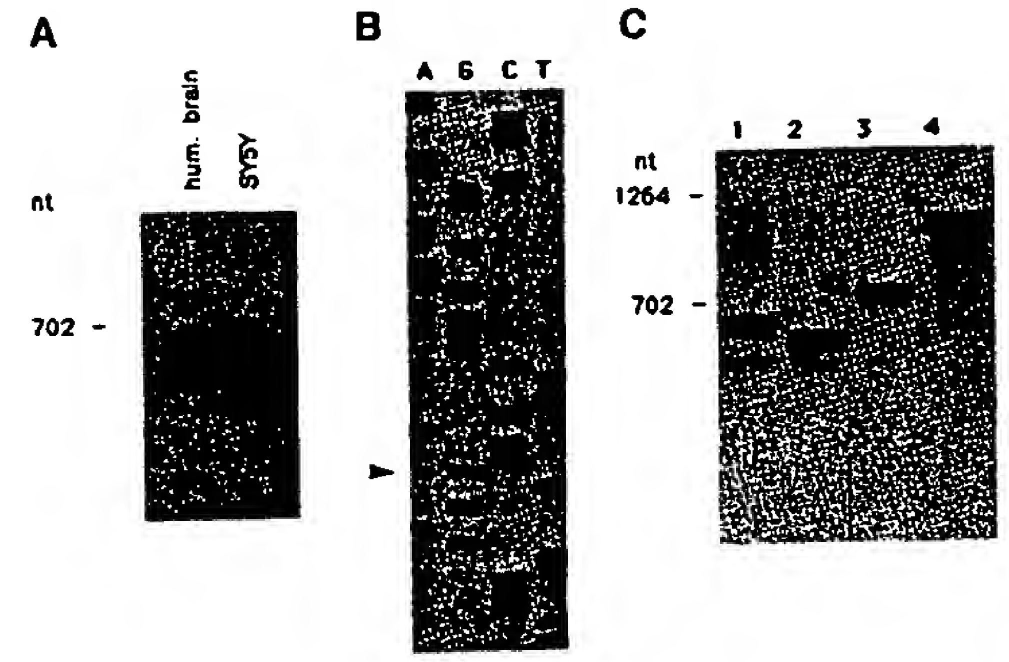

FIG. 1. (*A*) Southern blot of PCR products that encode the carboxyl-terminal half of the human (hum.) dopamine D_3-specific cDNA. The longer product is D_3-specific; the shorter product is D_{3nf}-specific. (*B*) Sequence of D_{3nf}-specific amplification product. Arrowhead indicates beginning of the discontinuity of the sequence of D_{3nf}-encoded cDNA compared with the D_3 sequence. (*C*) Southern blot of D_3-specific (lane 1) and D_{3nf}-specific (lane 2) PCR products using primer pair D3S5′/D33′. Lane 1 shows the PCR product from a cDNA template of cloned full-length human D_3-specific sequence. A 98-nt-shorter PCR product is amplified when cDNA obtained from parietal cortical tissue of schizophrenics is used as template (lane 2). The same cDNA yields a single PCR fragment of 887 nt when the D_{3nf}-specific sequence of *B* is used as a 3′ primer in conjunction with the 5′ primer D35′ (lane 3), and a product of 1110 nt is obtained when primer pair D35′/D33′ is used to amplify the full-length coding region of D_{3nf}-specific cDNA (lane 4). Size markers are derived from *Bst*E II-digested λ phage DNA (New England Biolabs).

(which recognizes the 5′ end of the D_3-specific sequence) a single product of 887 nt was amplified by PCR. This product (Fig. 1*C*, lane 3) is encoded by the 5′ portion of the D_{3nf}-specific cDNA, and its nucleotide sequence is identical to the corresponding nucleotide sequence encoding the 5′ portion of the dopamine D_3 receptor that has been reported (14). The same cDNA template led to the PCR amplification of a single 1110-nt-long fragment that spans an uninterrupted open reading frame when the primer pair D35′/D33′ (see *Materials and Methods*) was used (Fig. 1*C*, lane 4). Except for the deletion of 98 nt described above, the nucleotide sequence of the 1110-nt-long D_{3nf}-specific PCR product is identical to the sequence encoding D_3.

To test whether the shorter PCR fragment shown in Fig. 1*A* (D_{3nf}) is a PCR artifact, the same primer pair that amplified it was used for PCR with the cDNA clone encoding full-length D_3 as a template. Only a single product was amplified, which corresponds in size to the length of the D_3-specific product (Fig. 1*C*, lane 1). Furthermore, RNA was extracted from stably D_3-transfected COS-m6 cells, and the amplification of its first-strand cDNA also yielded only the D_3-specific PCR product (Fig. 2).

D_{3nf} mRNA differs from the previously reported D_3-specific cDNA only in the sequences encoding the carboxyl-terminal portion of the message (Fig. 3). To test whether D_{3nf} mRNA is derived from the D_3 primary transcript by alternative splicing, we amplified a genomic locus encoding D_3 by PCR with primers that flank the region of the differently utilized 98 nt in both D_3 and D_{3nf} mRNAs. The primer pair D3S5′(2)/D3S(2R) (see *Materials and Methods*) directs amplification of a 139-nt long genomic sequence that comprises D_3 mRNA sequences. A consensus sequence for 5′-(TGA:GU) splice sites that flanks at the 5′ end the 98 nt found only in the D_3 mRNA is present in this sequence. However, the "splice-junctional" sequence 5′-TGAGU-3′ found in D_{3nf} mRNA (Figs. 1*B* and 3) would predict that the downstream sequence 5′-GGA:GU-3′ functions as an "unusual" 3′ splice

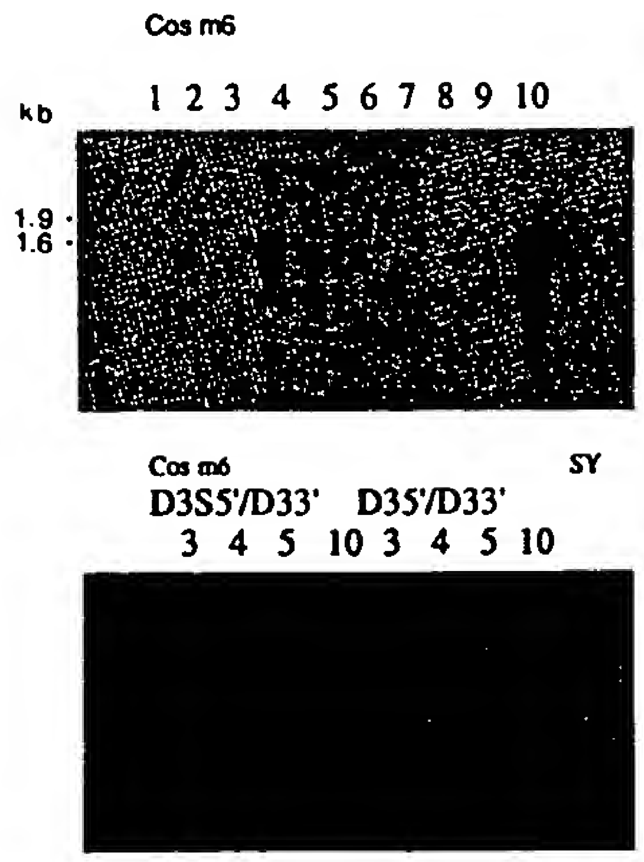

FIG. 2. (*Upper*) Northern blot of RNA extracted from stably D_3-transfected COS-m6 cells. A 1.5-kb-long mRNA species (in different amounts) was detected in clones 2–5 and 10 that hybridized to a ^{32}P-radiolabeled human D_3-encoding cDNA. (*Lower*) PCR amplification of RNA-derived cDNA of cell clones 3–5 and 10 with primers D3S5'/D33' (left) and with primers that direct amplification of the full-length coding region of D_3 (right; D35'/D33'). Only the D_3-specific mRNA could be amplified, whereas RNA extracted from SY5Y (SY) cells contains both D_3 and D_{3nf}-specific mRNAs (*Lower*, last lane).

site. In any case, in D_{3nf} mRNA, the exclusion of a 98-nt-long sequence found in the 3' portion of the putative third cytoplasmic domain in D_3 mRNA leads to a 1-nt frame-shift and, therefore, to a differently predicted carboxyl-terminal peptide sequence. Due to an earlier termination of the open reading frame, the predicted D_{3nf}-specific peptide is 58 amino acids shorter than the D_3 peptide and lacks D_3-typical transmembrane-spanning domains 6 and 7 (Fig. 3).

The mRNA extracted from several different parietal cortical tissues of schizophrenic patients was initially observed to contain only the D_{3nf}-specific sequence. To examine whether the processing of the D_3 mRNA differs in this brain region compared with nonschizophrenic brains, the distribution of both mRNAs was analyzed in different individual parietal cortical tissues (Brodmann areas 1, 2, 3, and 5) obtained postmortem from patients with chronic schizophrenia and from controls. The carboxyl-terminal portion of the D_3- and D_{3nf}-protein-encoding cDNAs was first amplified by use of primer pair D3S5'/D33' (see *Materials and Methods*; see Fig. 1*A*). In controls, both D_3- and D_{3nf}-specific cDNAs were simultaneously amplified in all tissues examined (Fig. 4*A*). However, in schizophrenic samples D_{3nf}-specific mRNA was almost exclusively expressed. Thus, D_{3nf} mRNA was detected in 18 out of 18 cases. In 16 of these cases no D_3 mRNA was detected (two samples expressed both mRNAs). Interestingly, preliminary studies on the expression of D_3 mRNA in the parietal cortex obtained postmortem from long-term-hospitalized patients with affective disorders revealed results similar to those found for schizophrenia. In contrast, the result obtained from a patient with chronic alcoholism/dementia is similar to results obtained with controls (see Fig. 4*A*, mixed).

The first nine D_{3nf}-specific cDNAs amplified from schizophrenic samples (Fig. 4*A*) were also cloned and sequenced. The nucleotide sequences were identical in all nine individuals; these nine samples were then studied in more detail. In a second series of experiments, D_3 and D_{3nf}-specific cDNAs were targeted selectively by PCR to control for possible kinetic differences in the amplification when both cDNAs are amplified simultaneously. A 5' primer (D35'; see *Materials and Methods*) was used in conjunction with either D_3-(RPD3) or D_{3nf}-specific (D3B3') 3' primers (Fig. 4*B*; see *Materials and Methods*). Both D_3 and D_{3nf}-cDNAs could be separately amplified in controls. In schizophrenia, D_{3nf} cDNA could be amplified in all samples, but only one of these samples expressed D_3 mRNA (Fig. 4*B*; compare sample S7 in Fig. 4 *A* and *B*).

Thus, there is a significant loss of D_3 mRNA expression in the parietal cortex of chronic schizophrenics. However, D_3 mRNA is not lost in general in postmortem brains of chronic schizophrenics. Fig. 5 shows that a consistent coamplification of D_3 and D_{3nf} cDNAs was observed in various different brain regions of controls. Also in most schizophrenic brain regions analyzed, both mRNAs were also found to be simultaneously expressed. However, no D_3 mRNA could be detected in the hippocampus, substantia nigra, and cerebellum. This expression pattern, however, needs further verification with a larger number of samples.

Fig. 6 shows the analysis of D_3 mRNA expression in the motor cortex (Brodmann area 4) of the same individuals for whom we analyzed mRNA expression in the parietal cortex. Interestingly, most of these schizophrenic samples lacked D_3 mRNA, in contrast with control or AD samples.

DISCUSSION

Our results suggest a selective loss of D_3 mRNA in the motor, primary somatosensory, and somatosensory association ar-

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ATG GCA TCT CTG AGT CAG CTG AGT AGC CAC CTG AAC TAC ACC TGT GGG GCA GAG AAC TCC   60
 M   A   S   L   S   Q   L   S   S   H   L   N   Y   T   C   G   A   E   N   S
ACA GGT GCC AGC CAG GCC CGC CCA CAT GCC TAC TAT GCC CTC TCC TAC TGC GCG CTC ATC  120
 T   G   A   S   Q   A   R   P   H   A   Y   Y   A   L   S   Y   C   A   L   I
CTG GCC ATC GTC TTC GGC AAT GGC CTG GTG TGC ATG GCT GTG CTG AAG GAG CGG GCC CTG  180
 L   A   I   V   F   G   N   G   L   V   C   M   A   V   L   K   E   R   A   L
CAG ACT ACC ACC AAC TAC TTA GTA GTG AGC CTG GCT GTG GCA GAC TTG CTG GTG GCC ACC  240
 Q   T   T   T   N   Y   L   V   V   S   L   A   V   A   D   L   L   V   A   T
TTG GTG ATG CCC TGG GTG GTA TAC CTG GAG GTG ACA GGT GGA GTC TGG AAT TTC AGC CGC  300
 L   V   M   P   W   V   V   Y   L   E   V   T   G   G   V   W   N   F   S   R
ATT TGC TGT GAT GTT TTT GTC ACC CTG GAT GTC ATG ATG TGT ACA GCC AGC ATC CTT AAT  360
 I   C   C   D   V   F   V   T   L   D   V   M   M   C   T   A   S   I   L   N
CTC TGT GCC ATC AGC ATA GAC AGG TAC ACT GCA GTG GTC ATG CCC GTT CAC TAC CAG CAT  420
 L   C   A   I   S   I   D   R   Y   T   A   V   V   M   P   V   H   Y   Q   H
GGC ACG GGA CAG AGC TCC TGT CGG CGC GTG GCC CTC ATG ATC ACG GCC GTC TGG GTA CTG  480
 G   T   G   Q   S   S   C   R   R   V   A   L   M   I   T   A   V   W   V   L
GCC TTT GCT GTG TCC TGC CCT CTT CTG TTT GGC TTT AAT ACC ACA GGG GAC CCC ACT GTC  540
 A   F   A   V   S   C   P   L   L   F   G   F   N   T   T   G   D   P   T   V
                                                                    D3S5'
TGC TCC ATC TCC AAC CCT GAT TTT GTC ATC TAC TCT TCA GTG GTG TCC TTC TAC CTG CCC  600
 C   S   I   S   N   P   D   F   V   I   Y   S   S   V   V   S   F   Y   L   P
TTT GGA GTG ACT GTC CTT GTC TAT GCC AGA ATC TAT GTG GTG CTG AAA CAA AGG AGA CGG  660
 F   G   V   T   V   L   V   Y   A   R   I   Y   V   V   L   K   Q   R   R   R
AAA AGG ATC CTC ACT CGA CAG AAC AGT CAG TGC AAC AGT GTC AGG CCT GGC TTC CCC CAA  720
 K   R   I   L   T   R   Q   N   S   Q   C   N   S   V   R   P   G   F   P   Q
CAA ACC CTC TCT CCT GAC CCG GCA CAT CTG GAG CTG AAG CGT TAC TAC AGC ATC TGC CAG  780
 Q   T   L   S   P   D   P   A   H   L   E   L   K   R   Y   Y   S   I   C   Q
GAC ACT GCC TTG GGT GGA CCA GGC TTC CAA GAA AGA GGA GGA GAG TTG AAA AGA GAG GAG  840
 D   T   A   L   G   G   P   G   F   Q   E   R   G   G   E   L   K   R   E   E
AAG ACT CGG AAT TCC CTG AGT CCC ACC ATA GCG CCT AAG CTC AGC TTA GAA GTT CGA AAG  900
 K   T   R   N   S   L   S   P   T   I   A   P   K   L   S   L   E   V   R   K
CTC AGC AAT GGC AGA TTA TCG ACA TCT TTG AAG CTG GGG CCC CTG CAA CCT CGG GGA GT G 960
 L   S   N   G   R   L   S   T   S   L   K   L   G   P   L   Q   P   R   G   V
D3nf A   T   S   G   E   E   G   N   P   N   G   G   H   C   A   W   G   L   H   C
    CCA CTT CGG GAG AAG AAG GCA ACC CAA ATG GTG GCC ATT GTG CTT GGG GCC TTC ATT GTC 1020
D3   P   L   R   E   K   K   A   T   Q   M   V   A   I   V   L   G   A   F   I   V
     L   L   A   A   L   L   L   D   P   C   S   Q   Y   P   L   P   D   M   P   R
    TGC TGG CTG CCC TTC TTC TTG ACC CAT GTT CTC AAT ACC CAC TGC CAG ACA TGC CAC GTG 1080
     C   W   L   P   F   F   L   T   H   V   L   N   T   H   C   Q   T   C   H   V
     V   P   R   A   L   Q   C   H   D   M   A   G   L   R   E   *
    TCC CCA GAG CTT TAC AGT GCC ACG ACA TGG CTG GGC TAC GTG AAT AGC GCC CTC AAC CCT 1140
     S   P   E   L   Y   S   A   T   T   W   L   G   Y   V   N   S   A   L   N   P
    GTG ATC TAT ACC ACC TTC AAT ATC GAG TTC CGG AAA GCC TTC CTC AAG ATC CTG TCT TGC 1200
     V   I   Y   T   T   F   N   I   E   F   R   K   A   F   L   K   I   L   S   C
                                                                            D33'
    TGA GGG AGC A
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FIG. 3. Nucleotide sequence and deduced amino acid sequence of human D_3 and D_{3nf}-encoding cDNA. The 98 nt deleted in D_{3nf} mRNAs are boxed. The carboxyl-terminal peptide sequences for D_{3nf} and D_3 are given above and below the nucleotide sequence, respectively. Positions of primers used for PCR amplifications shown in Fig. 4*A* are indicated.

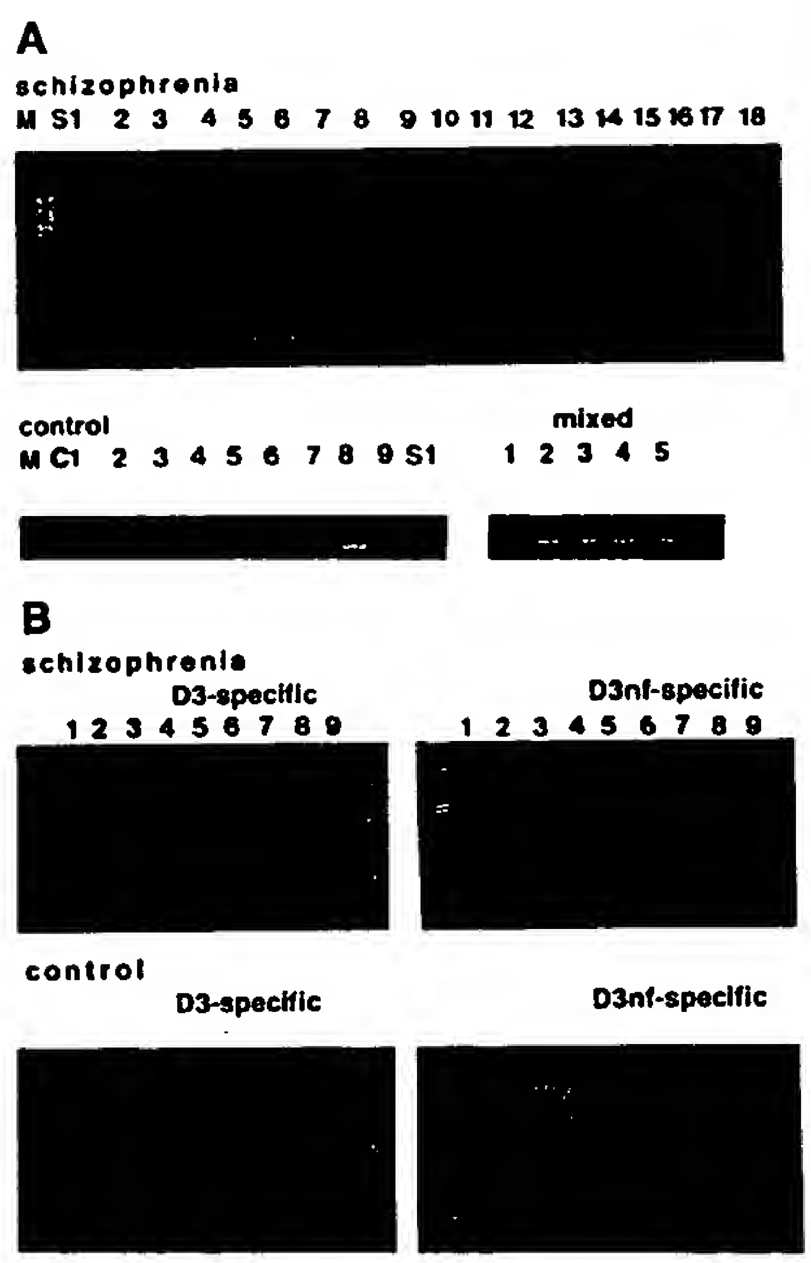

FIG. 4. (*A*) Visualization of ethidium bromide-stained PCR products of parietal cortical cDNAs on agarose gels. One case of chronic alcoholism/dementia (female, 70 yr, PMI: 440 min) (lane 1) and four cases of bipolar affective disorders (two females, two males; 58, 59, 70, and 70 yr; PMIs: 737, 216, 401, and 410 min) (lanes 2–5) are also shown (mixed). The PCR amplification of the carboxyl-terminal portion of the coding regions of D_3- and D_{3nf}-specific cDNAs was directed by primer pair D3S5′/D33′. (*B*) Separate amplification of D_3 and D_{3nf} cDNAs with specific 3′ primers (RPD3 and D3B3′) and the 5′ primer D35′. The amplified products correspond to the 5′ three-fourths of the coding regions for D_3 and D_{3nf}. Size markers (M) are derived from *Bst*EII-digested λ phage DNA.

eas of cortices obtained postmortem from patients with chronic schizophrenia (and possibly also patients with chronic affective disorders). Expression of D_{3nf} mRNA, however, is unaffected in these regions.

With the exception of a 98-nt-long deletion, D_{3nf} mRNA is identical to D_3 mRNA. If this 98-nt-long sequence (present in D_3 but missing in D_{3nf}) is defined as an intron during the D_{3nf}-specific posttranscriptional processing, the expression of D_{3nf} mRNA is regulated by alternative splicing of the D_3 primary transcript. Although a consensus sequence for 5′ splice sites is flanking this putative intron at its 5′ end, the 3′ cleavage site is predicted to be GA:N. This cleavage site would be unusual because 3′ splice sites are generally highly conserved and almost invariantly AG-dependent. If cleavage occurs at the consensus AG:N sequence, one would predict the exclusion of a 99-nt (rather then a 98-nt)-long intron, which would not shift the open reading frame (see Fig. 3). However, careful sequencing of many D_{3nf} clones in both orientations does not support this scenario. Therefore, three possibilities could account for the generation of D_{3nf} mRNA: (*i*) It results from the D_3-specific primary transcript by alternative splicing and uses an unusual 3′ splice site. (*ii*) The alternatively cleaved 3′ splice site is localized 1 nt downstream from the above predicted cleavage site and is AG:UG (see Fig. 3), and a single guanine is edited after cleavage. (*iii*) D_{3nf} is the transcript of a different, not-yet-identified gene. In any case, the D_{3nf} mRNA sequence would encode a protein that is 58 amino acids shorter then the D_3 peptide (Fig. 3), and

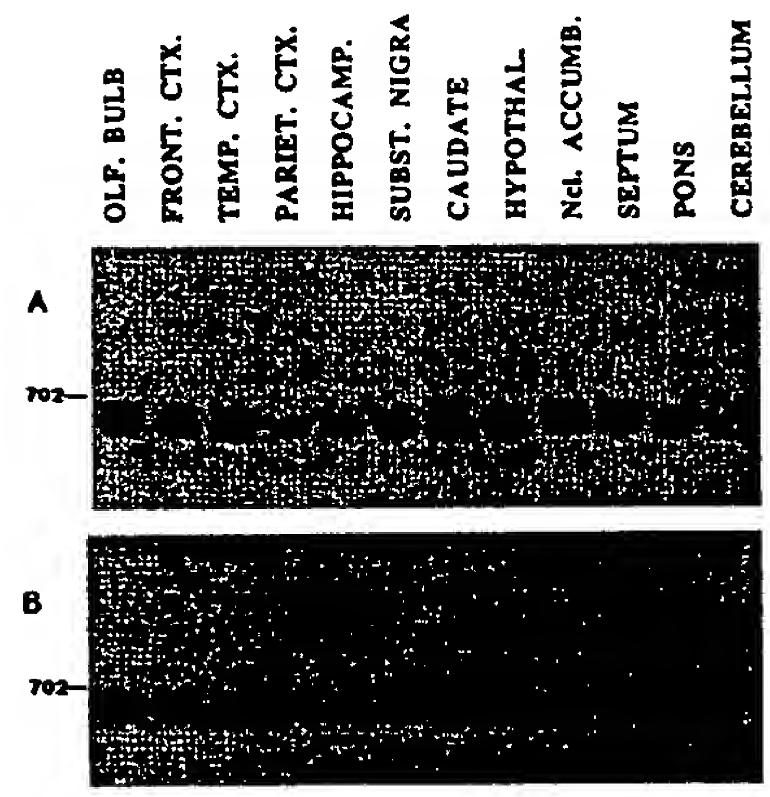

FIG. 5. Southern blot of PCR products that encode the carboxyl-terminal halves of D_3 and D_{3nf}. (*A*) Schizophrenia. (*B*) Controls. In a few cases it was necessary to pool tissues from several individuals of the same diagnostic group for RNA extraction [olfactory bulb (OLF. BULB): four schizophrenia, six controls; frontal cortex (FRONT. CTX.; Brodmann area 10): two schizophrenia, one control; temporal cortex (TEMP. CTX.; Brodmann area 21): two schizophrenia, one control; parietal cortex (PARIET. CTX., Brodmann areas 1, 2, 3, and 5): one schizophrenia, one control; anterior portion of the hippocampus (HIPPOCAMP.): two schizophrenia, one control; substantia (SUBST.) nigra: two schizophrenia, one control; head of the caudate: two schizophrenia, one control; hypothalamus (HYPOTHAL., level of mammilary bodies): two schizophrenia, one control; nucleus accumbens (Ncl. ACCUMB.): three schizophrenia, three controls; septum: three schizophrenia, three controls; subcochlear level of the pons: two schizophrenia, one control; cerebellar cortex: two schizophrenia, one control. Size markers are derived from *Bst*E II-digested λ phage DNA.

it is unlikely that D_{3nf} compensates functionally for the loss of D_3 receptors because its different transmembrane topology separates this putative protein from typical G protein-coupled receptors with seven transmembrane-spanning domains. In addition, we failed to detect dopaminergic-specific

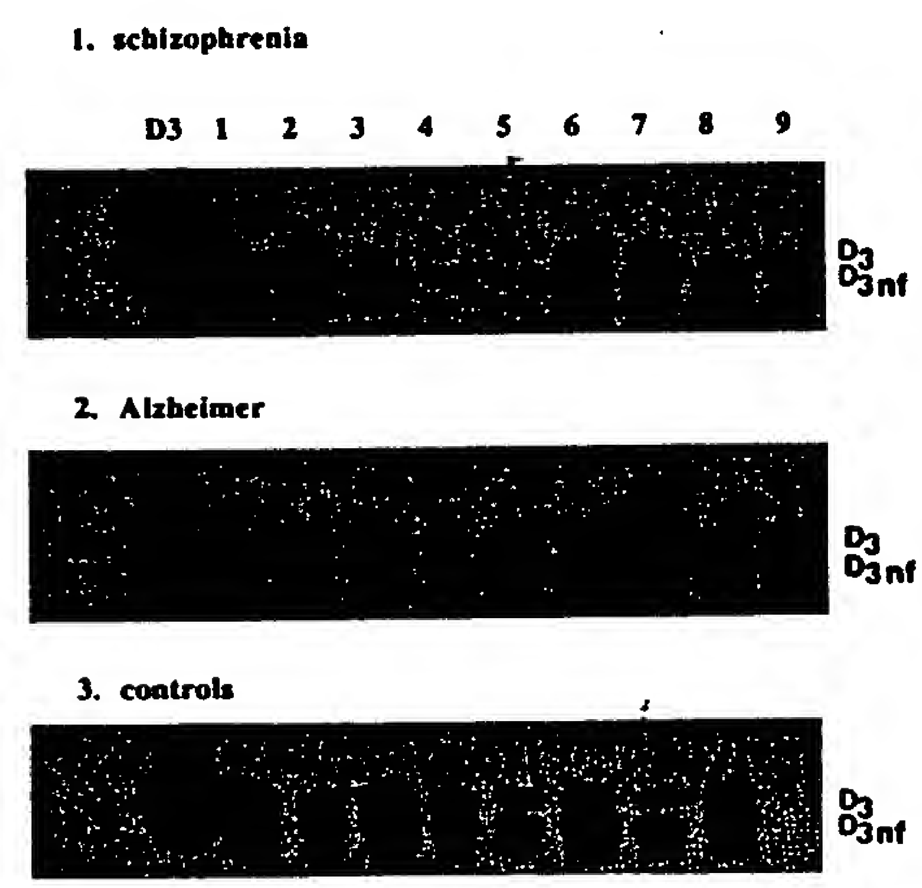

FIG. 6. Southern blot of PCR-amplified D_3 and D_{3nf} cDNAs from the motor cortex. Primer pair D3S5′/D33′ was used. The control lane (marked as D3) shows the D_3-specific amplification of cDNA obtained from RNA of stably D_3-transfected COS-m6 cells (see Fig. 2). The nature of the third and shortest hybridizing PCR product seen predominantly in the schizophrenia and AD samples is presently unclear and is, most likely, a PCR artifact.

high-affinity binding in membranes from transfected CHO cells that expressed D_{3nf} mRNA (data not shown).

The observation that both D_3 and D_{3nf} mRNAs can also be amplified from RNA-derived cDNA templates of cultured neuronal SY5Y cells indicates that D_{3nf} mRNA is not uniquely generated postmortem. Furthermore, the differences found in D_3 mRNA expression between schizophrenia (and possibly also affective disorders) and AD or controls cannot be explained by differences in the ages of the individuals examined, their ethnic background, or the PMIs because these parameters were similar in all three diagnostic groups.

Many variables may account for the apparent lack of D_3 mRNA expression in certain cortical regions of patients with chronic psychosis. One possibility is that the selective loss of D_3 mRNA is a region-specific outcome of neuroleptic treatment. At present, however, we have found no correlation between the histories of neuroleptic treatment of the individuals examined in this study and the expression pattern of D_3. A further possibility is that the selective loss of D_3 mRNA in some cortical regions is an outcome of a long-lasting psychotic disorder (schizophrenia and affective disorders) with the resultant need for long-term hospitalization. The motor cortex is a region known to be affected by various forms of deprivation, and in this region most schizophrenic samples lack D_3 mRNA. This D_3 lack does not appear for controls and is not found for patients with AD, a disease being, on average, of much shorter duration then the cases of schizophrenia studied here.

The presence of other shorter variants of the human D_3-receptor-encoding cDNA has been reported (14, 20). All these mRNA variants were identified by use of the powerful PCR amplification method. We have performed S1 nuclease protection assays with RNA extracted from the parietal cortex of our control brains and could detect both mRNA species by this method. Results from these preliminary experiments revealed that the small amount of D_3 mRNA is still severalfold more than D_{3nf} mRNA. The function of D_{3nf} (if any) is, at present, not clear.

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